Drug nanosuspensions: A ZIP tool between traditional and innovative pharmaceutical formulations

DRUG NANOSUSPENSIONS: A ZIP TOOL BETWEEN TRADITIONAL AND INNOVATIVE PHARMACEUTICAL FORMULATIONS

Abstract

Introduction. A drug nanosuspension is a versatile technological approach to formulating poorly water soluble molecules consisting of 100 % pure drug nanocrystals with sizes in the nano-scale range. Nanosuspensions can be obtained with bottom-up and top-down methods or by their combination. They can enhance the bioavailability of drugs via various administration routes. Due to their sizes, nanosuspensions can be also considered to be drug delivery nanotechnologies.

Area covered. This review focuses on the state of the art of the nanocrystal-based formulation. It describes characteristics, design parameters, preparation methods, stability issues, as well as specific applications via various administration routes. The potential of nanocrystals as nanomedicine formulation is also reported.

Expert opinion. Many drug delivery systems have been developed to increase the bioavailability of drugs and to decrease adverse side effects, but few can be industrially manufactured. Nanocrystals can be considered versatile means with which to combine traditional and innovative drug formulations. Indeed, they can be used in many pharmaceutical processes as such, or as nano-scaled carriers. Engineered surface nanocrystals have been recently proposed for stability and targeting purposes.

Keywords: bioavailability, drug delivery, nanomedicine, nanosuspensions, pure drug nanocrystals

1. **Introduction**

Currently, poor water solubility is one of the most challenging issues in the development of new drugs, considering that about 40% of the new chemical entities (NCEs) are practically insoluble in water (1). Drug solubility plays a significant role during drug administration, especially for oral and parenteral formulations (2, 3) because modifying the solubility makes it possible to achieve the concentration of the drug in the blood required to obtain a pharmacological response (4). Indeed, orally administered poorly water soluble molecules often need high doses to reach therapeutic plasmatic concentrations, with the possibility of causing serious dose-related side effects.

Drug biopharmaceutical properties are described in the Biopharmaceutics Classification Systems (BCS) developed by Amidon (5-9), in which drugs are classified into four classes according to their solubility and permeability properties (10). Class I includes molecules with both high solubility and high permeability; while class II comprises compounds with low solubility and high permeability. Class III includes molecules with high solubility but low permeability, and class IV compounds in which both poor solubility and poor permeability limit drug absorption.

Consequently, only class I molecules do not entail problems with oral absorption. There are several approaches to improving drug solubility (11). They are based on the modification of the physico-chemical drug properties, on the use of suitable functional excipients, or on the application of various technological strategies. Schematically, solubility enhancement methods may be classified as follows:

a. Modification of the drug medium (e.g. pH, solvent/cosolvent mixture, surfactants) (12, 13) .
b. Reservoir systems (e.g. cyclodextrin inclusion complexes, lipid formulation) (14, 15)
c. Solid state modification (e.g. amorphous solid dispersion, size reduction, such as micronization, nanosizing, particle engineering)(16, 17).
d. Nanotechnology drug delivery system (e.g. vesicles, nanoparticles, nanosponges) (18-20).

Among these approaches, the possibility of developing pure drug nanosuspensions by means of nanosizing methods is becoming increasingly important, given their versatility and capacity to overcome some formulation drawbacks, such as the large use of additives (21).

Therefore nanosuspensions consist of 100% drug crystals with sizes in the submicron range, usually dispersed in liquid media, either aqueous or non-aqueous, and stabilized by surfactants and polymers (22).

The addition of stabilizer is necessary to ensure an adequate stability of the formulation, as the nanoscaled sizes leads to a larger surface area than that of conventional suspensions.

Nanocrystals can be classified as belonging to a first and second generation. The first generation consists of pure drug nanosuspensions obtained with bottom-up and top-down techniques. The second generation includes nanocrystals with improved properties achieved with combination technologies. Finally, engineered nanocrystals can be considered the third generation.

Size reduction represents an advantage for poorly water soluble compounds because its leads to an enhanced saturation solubility and dissolution rate with the relative improved drug bioavailability (17). Recently this formulation strategy has been proposed also for medium soluble compounds in order to improve their dermal delivery (23). The rationale for this new type of dermal formulation is that nanocrystals can act as fast dissolving reservoirs increasing the drug's saturation solubility. Moreover, the nanometer sizes can favour nanocrystal accumulation in the hair follicles.

Surface-functionalized nanocrystals can be obtained from either the coating or the conjugation of targeting ligands and they are under investigation as drug nanocarriers with the potential to target specific organs.

The purpose of this review is to describe nanosuspensions composed of drug nanocrystals from a technological point of view, with examples of their application via different routes of administration, as well as their use as innovative nanomedicine for drug delivery (Figure 1) (24-26).

1. **Physico-chemical background of nanosuspensions**

Size reduction leads to an increased surface area and greater dissolution velocity of a solid, particularly from microparticles to nanoparticles, according to the Noyes-Whitney equation and to the subsequent Nerst-Brunner and Levich modification (27, 28).

Moreover, particle size reduction can result in the increased saturation solubility of a compound.

In the case of conventional coarse powders, the saturation solubility is a constant which depends on the substance, the dissolution medium and the temperature (24). Indeed, the saturation solubility of nanoparticles is also a function of the particle size (29), as postulated by by the Ostwald- Freundlich equation.

The Kelvin equation is another mathematical law useful for describing drug behaviour . It considers the transition of a molecule from a liquid phase, such as a droplet, to a gas phase; and the same thereotical principle can be applied to the molecule's transition from a solid phase, such as nanocrystals, to a liquid phase, for example the dispersion medium (24).

The increased saturation solubility of drug nanocrystals can enhance the concentration gradient between, for example, gut lumen and blood. It consequently improves the absorption by passive diffusion (30) of a drug poorly adsorbed in the gastro-intestinal tract.

Another distinctive characteristic of nanocrystals is their greater adhesiveness to biological membranes and tissues, such as the intestinal epithelium. This behaviour has already been observed in various nanoparticle systems (21, 31), resulting in a prolonged residence time in the gastro-intestinal tract (32, 33). The drug's nanosuspension adhesiveness can be exploited in various administration routes other than the oral one, such as topical, in order to improve local and dermal drug delivery. The adhesiveness of nanocrystals can be further improved by coating their surface with mucoadhesive polymers. For this purpose, chitosan and Carbopol® have been extensively investigated (34-36).

Recently, surface coated nanocrystals have also been advocated for site-specific targeting (21, 37, 38).

2. Methods of preparation

In regard to the preparation of nanosuspensions, two main approaches, either at lab-scale or on an industrial scale, can be used: bottom-up or top-down technologies (Figure 2). The former are classified as assembling methods which start with molecules in solution and aggregate them into solid particles, which can be crystalline or amorphous. By contrast,

top-down technologies are disintegrating methods which start from a coarse material to obtain a nanosized formulation.

2.1 Bottom-up technologies

Bottom-up technologies can be divided between methods based on two different strategies: solvent precipitation and controlled evaporation (39, 40).

The former approach comprises a series of steps: chemical reaction (and the subsequent supersaturation), nucleation, solute diffusion and particle growth (39). These processes are exploited with different techniques, such as nanoprecipitation or solvent displacement (41), pharmaceutical hydrosols (42), high-gravity controlled precipitation (HGCP) (43), flash nanoprecipitation (44, 45), supercritical fluid (SCF) technology (46) and sonoprecipitation (47). Recently Tran et al. produced isradipine nanosuspensions showing that amplitude and time of ultrasonication can markedly affect the sized of the nanocrystals (48).

Nanoprecipitation is a straightforward process: a lipophilic drug is dissolved in an organic solvent, and this solution is then added to a non-solvent for the drug (generally water) in which the polar solvent is miscible. This leads to a phase separation with the formation of solid drug particles. The organic solvent can be eliminated by evaporation. The achievement of very small and monodisperse drug nanocrystals is due to the Ouzo effect (41, 49, 50). Rapid addition of the solution to the non-solvent leads to rapid drug supersaturation, and to the formation of an ultrafine amorphous (predominantly) or crystalline drug. This method involves nuclei formation and crystal growth which are mainly dependent on temperature.

The anti-solvent method is suitable and cost-effective for lab-scale formulation studies, but it is not convenient for industrial-scale production, which needs more controllable parameters and equipment.

Nanoparticle growth is the main parameter affecting the nanosuspension's stability. In order to control the process, a wide range of additives for anti-solvent precipitation, including polymers (e.g. polyvinyl alcohol, cellulose ethers, chitosan, agar, pectin, and

gelatine), sugar (e.g. trehalose), and surfactants (e.g. poloxamers, partial fatty acid esters, polyoxyethylene fatty alcohol ethers, and phospholipids), have been investigated (39).

Bottom-up methods also include technologies based on the controlled evaporation of droplets, such as the spray drying technique (51), the aerosol flow reactor method (52) and electrospray (53). These approaches are based on evaporation of the solvent from droplets obtained from the atomization of a drug solution. The rapid solvent elimination produces nanometer-sized particles of the drug. Moreover, the preparation of drug nanosuspensions by a solvent quenching technique has also been investigated: briefly, by emulsifying an organic solution of the drug in an aqueous solution of a stabilising agent it was possible to obtain solid particle formation by rapid displacement of the solvent from the internal to the external phase (54, 55). Interestingly, it is possible to control the nanocrystal's size by controlling the size of the emulsion droplets.

2.2 **Top-down technologies**

Using these technologies, a coarse material can be nanosized by applying forces. The two main top-down techniques are pearl milling and high pressure homogenization.

The former was developed by Liversidge (16, 39, 56, 57). Generally, pearl mills consist of a milling chamber filled with fine milling pearls or media. The container can be static or otherwise: in the former milling technique coarse material is moved by a stirrer; in the latter, the complete milling chamber is moved, also stirring the milling pearls. Milling media can consist of various materials, such as zircon oxide, steel, glass and hard polystyrene.

A coarse powder can be processed with two different milling approaches: dry milling and wet milling. In the former method, the rough material is nanosized in the milling chamber by using milling media to obtain a finer product. In the latter, the milling container is filled with a pre-suspension obtained from a coarse powder dispersed in an anti-solvent. Generally, dry milling is not sufficient to obtain submicron particles. Therefore the wet milling process is the one most frequently applied (58).

Milling time, speed, temperature and drug amount are critical parameters to take into account when using the milling technique. For example, a complete milling process can last

from hours to several days depending on the desired results (38). Evidently, this can be considered a significant limitation of milling technology, especially from an industrial perspective (57).

One of the main drawbacks associated with pearl milling technology is erosion from the milling material or from the chamber during the milling process. Various parameters can affect the degree of erosion, such as milling time and initial material conditions. Usually, in order to deal with this problem, the milling chamber and the milling media are covered with specific materials, such as various plastics.

The easy scalability from laboratory-scale to industrial-scale and its cost effectiveness are the most significant advantages of this technique.

Two products based on the pearl-milling technology are available on the market: Rapamune® and Emend®.

Another important top-down technique is high pressure homogenization, which exploits high energy processes to reduce particle dimensions. There are two important homogenization technologies: microfluidizer technology and piston gap homogenization technology, which can be performed in water or in water mixtures and in non-aqueous media (24).

The jet-stream principle is the basis of the microfluidizer technology (Microfluidics™ Inc., USA.). In this case, pure drug particles in suspension can be nanosized by making two streams of liquid collide. Crystal reduction is obtained by two different mechanisms consisting of particle collision and cavitation. The main drawback for industrial-scale production is the high number of cycles, from 10 to 500, required during the nanosizing process (59). Insoluble Drug Delivery-Particles (IDD-P™) technology is based on the jet stream principle and it belongs to SkyePharma Canada Inc.

In regard to piston gap homogenization techniques, two main technologies based on this principle have been developed by Müller: Dissocubes® (SkyePharma PLC) and Nanopure® (Abbot) (60). In the Dissocubes® method an aqueous pre-suspension composed of a drug powder and a surfactant is forced by a piston under pressure (typically 1500–2000 bar) through a tiny homogenization gap, whose size range is between 5 and 20 μm. The high

streaming velocity inside the homogenizer causes an increase in the dynamic pressure and a reduction in the static pressure below the vapour pressure of the aqueous phase, as described by the Bernoulli equation. This mechanism induces water to start boiling and form gas bubbles, which collapse instantaneously when the liquid leaves the homogenization gap. Shock waves caused by cavitation can contribute to the disintegration of the crystals in suspension.

The efficiency of the piston gap homogenization technique depends on several critical parameters, such as homogenizer pressure, number of homogenization cycles, and hardness of the drug (60).

The Nanopure® technology is an alternative approach based on the piston-gap homogenizer exploiting a non-aqueous pre-suspension, in which the liquid medium can be composed of oils, PEGs, or mixtures of water and organic solvents (i.e. glycerol–water, ethanol–water). These media are characterized by a low vapour pressure which reduces or excludes the cavitation phenomenon. Therefore crystal disintegration takes place due to shear forces, particle collisions and turbulences induced by the homogenizer. Moreover, the Nanopure® process can occur at low temperatures, so temperature-sensitive drugs can be processed. Nanosuspensions obtained with this technology are commonly used to fill capsules.

2.3 **Combination Technologies**

Generally, these techniques consist in the combination of bottom-up and top-down approaches.

The NANOEDGE™ technology (Baxter) involves a first pre-treatment step consisting in the precipitation method, with a subsequent annealing step, i.e. high pressure homogenization (61). During the annealing step, several types of energy (heat, mechanical) can be applied to convert matter into a more stable form in order to prevent the growth of the nanocrystals obtained with the precipitation approach. The main drawback of this technology concerns the use of organic solvents during the precipitation step and the subsequent necessity to remove them, which is a time-consuming and expensive procedure from an industrial perspective.

Interestingly, clarithromycine nanocrystals were obtained by a method comprising precipitation-lyophilization and homogenization (62). This three combination method, called PLH, is particularly suitable for compounds that request high pressure and more cycles of homogenization for reducing their sizes. The pre-treatment by precipitation can reduce the initial particle sizes of the drug and then the lyophilisation step can provide more porous and friable particles. Therefore the PLH combination method can permit to achieve rapidly nanocrystals decreasing the homogenization cycles.

The smartCrystal® technology, owned by Abbott and marketed by Soliqs, was developed as a "tool box" of various combination processes (63, 64). An improved *in vivo* performance can be obtained through several process variations. H42 is an example of smartCrystal® technology: this process is a combination of spray-drying and high pressure techniques. Instead, in the H96 process, lyophilization and high pressure homogenization are combined in order to obtain nanocrystals with sizes less than 100 nm.

Despite of the preparation method, the quality and the efficacy of a nanosupension formulation must be assessed, and a detailed chemical and physical characterization is required. Table 1 reports the key features of drug nanocrystals in suspension relative to the principal techniques available to characterize them. The stability of nanosuspensions will be discussed in the next paragraph.

3. Stabilization of nanosuspensions

The stability of nanocrystals is a critical parameter for formulations of this type. The nanometer-sized dimensions of drug nanocrystals may be the main drawback of these formulations because they give rise to stability issues and also to potential nanotoxicity after their administration (65-67).

Stability issues can be classified as either chemical or physical. Whilst the former are drug-specific issues related to each molecule with its specific functional groups, the latter are formulation-specific limitations depending on the dosage forms. Instability problems with nanosuspensions may arise at any phase of the product life cycle: from the preparation of

the finished product to its distribution and storage (67). Generally, a solid is more stable than a solution; consequently, the chemical degradation is limited.

In vitro stability

The main physical stability issues involved are comparable to those of traditional pharmaceutical suspensions, including sedimentation/creaming, agglomeration, crystal growth and modification of the crystallinity state.

The settling velocity of small spherical particles in a fluid is described by Stoke's Law and depend mainly by the radius of the dispersed nanoparticles, the density of the medium and the viscosity of the fluid medium. Indeed, the nano-scaled dimensions of nanocrystals can ensure a long-term stability influencing the sedimentation rate. Anyway, stability issues may also arise from agglomeration phenomena, which can lead to a rapid particle settling producing as a consequence inconsistent dosing.

Agglomeration is mainly caused by the nanoparticles' large surface area, related to their nano-structure, which determines an increase of surface energy that might cause attraction of nanoparticles. Nanocrystal aggregation leads to a reduction in the surface area of the system, decreasing consequently the dissolution velocity and modifying the drug bioavailability. Another significant stability issue affecting colloidal suspensions is the crystal growth usually known as Ostwald ripening, which arises from the particles' solubility dependence on their size.

A drug concentration gradient is generated between small and large particles due to the high saturation solubility affecting small particles. Consequently, molecules diffuse from the small particles' surfaces at higher concentration to layers surrounding larger particles with lower drug concentration. Drug crystallization onto the large particles is then induced by the supersaturated solution around them causing particle grow.

Suspensions of drug nanocrystals with a narrow particle size distribution can prolong the long-term stability of formulations, reducing the drug concentration gradients between smaller and larger particles. Moreover, stabilizer addition, temperature and mechanical

agitation can affect Ostwald ripening. The layer-by-layer (LbL) self-assembly coating of nanocrystals was recently proposed as a stabilization approach to overcome the Ostwald ripening instability (Strydom, Powder Technology, 2014). The process consists of the successive deposition of polyelectrolytes of altenating polarities to form a nanoshell around the solid particles by electrostatic interaction. Theeffect of the coating was evaluated with four different model drugs: furosemide, isoxyl, rifampicin and paclitaxel to prove the versatility of the mechanisms. This manufacturing process produced stable drug nanocrystals with a reduced dissolution rate according to the coat thickness…

Finally, the crystalline state is an another important parameter to be taken into account. It describes the nature of the drug nanocrystals. Commonly, drug particles can have a crystalline structure or an amorphous state. Transformation between these two molecular states is a common issue affecting a suspension's stability. By applying forces to reduce crystal sizes, an amorphization of the sample can occur (Willet, Mole pharmaceutics, 2008) . For amorphous solids the risk is that the drug crystallizes during storage, changing formulation characteristics. Most amorphous particles are unstable because of their high energy, and they tend to convert to a crystalline state with a lower energy.

Therefore the control of crystallinity is mandatory during the production processes. It is generally well established that top-down and bottom-up techniques can produce partially or completely amorphous (especially with the precipitation approach) nanosuspensions. The presence of stabilizer can favour the formation of amorphous layer on nanocrystal surfaces. Kayart and van der Mooter investigated the effect of media milling on generation of amorphous in the presence of HPMC using cinnarizine and naproxen as model drugs. The media milling in the presence of the polymer can affect the solid state of nanocrystals due to the drug-polymer interactions. The study showed an amorphization of naproxen, while no amorphous fraction was observed in cinnarizine sample which is not soluble in HPMC. Therefore drugs that are more soluble in the stabilizer can form amorphous in a greater extent .

Wu (Langmuir, 2007) showed that nanocoatig can be a way to inhibit the surface crystallization of amorphous indomethacin, considering that amorphous solid may

crystallize faster at the interface for the higher molecular mobility at the free surface than in the bulk. The small thickness of the mobile surface layer suggests that it could be immobilized by a coating. indomethacin nanocrystals were coated with a polymer coating formed by a layer-by-layer assembly of opposite charges polyelectrolytes, such as cationic poly(dimethyl(diallyl)ammonium chloride), PDDA and anionic sodium poly(stirenesulfonate), PSS, or by an ultrathin coating of gold. The results showed that the surface crystallization of amorphous indomethacin was inhibited. The coating also inhibited the crystal growth of the nanocrystals stabilizing the nanosuspension.

Moreover polymorphism transformation must also be evaluated during nanosuspension formulation development. The conversion of different polymeorph is a possible phase transition during milling in the absence of water or other liquid media. It was observed that generally in the presence of water amorphizationis difficult probably because water can act as plasticizer lowering the glas transition temperature and favouring a rapid crystallization of the material (Willord Carbohydrted Research).

The addition of ionic or non-ionic surfactants or polymers as stabilizers are the most common approaches to obtain a stable nanosuspensions exploiting electrostatic repulsion and steric stabilization. In regard to electrostatic stabilization, the effects of electrolytes on colloid stability is described by DLVO theory, which considers the interaction between electrostatic repulsive forces and van der Waals attractive forces on particles in suspension. The sum of repulsion potential and attraction potential from the van der Waals forces results in the total potential energy of the interaction between nanoparticles.

The electrical potential is commonly termed the Zeta potential (ζ). It is one of the most significant parameters used to describe suspension stability.

Electrostatic stabilization is obtained by the adsorption of ionic surfactants on the nanocrystal surface, such as sodium dodecyl sulfate (SDS), soy lecithin, sodium cholate and sodium docusate.

Steric stabilization is achieved by the adsorption of polymers.

The non-ionic surfactants and polymers generally employed are Pluronic®, Tween® 80, polyethylene glycol (PEG), polyvinyl alcohol (PVA) polyvinylpyrrolidone (PVP), and cellulose ether derivatives such as hydroxypropyl cellulose (HPC) and hydroxypropyl methylcellulose (HPMC) (67).

Van Eenderbrugh et al investigated 13 structurally different stabilizers at three different concentration to stabilize 9 different compound nanocrystals. The results showed that cellulose derivatives displayed poor stabilization due to their viscosity-limited concentration, while Polysorbate 80 and TPGS showed the best stabilizing performance (68).

To obtain the nanoparticle steric stabilization the addition of amphiphilic non-ionic stabilizers plays an important role . These molecules has two parts: an anchor segment, which is absorbed into the drug nanocrystals and strongly interacts with them, and a tail segment which prolongs into the bulk medium. The solvation effect, strong enthalpic interaction between the stabilizer and the solvent, is mandatory to achieve steric stabilization and to avoid particle agglomeration in the suspension. Temperature and the stabilizer concentration are the most significant limiting factors affecting the solvation effects on the stabilizer molecule, as well as the interaction between particles and stabilizer. Appropriate intermolecular interactions between the drug and the polymer molecules are a key factor in preventing aggregation.

The main disadvantage of the steric stabilization mechanism is that the stabilizer choice is strongly dependent on drug-specific molecular structure. Therefore current surfactant screening methods are based on empirical observations. However, steric stabilization is generally the first choice because it is less susceptible to electrolytes in the gut or blood (59).

Recently Ochi evaluated a series of hydrophilic polymers as aggregation inhibitors of meloxicam nanocrystals (69). The crystal growth inhibition in a supersaturated drug solution was determined in order to select the most suitable macromolecules. The screening rationale is that the polymer that inhibited crystal growth in a supersaturated solution is able to prevent aggregation (70). PVP K30 showed the best performance as

stabilizers. Then PVP/meloxicam nanocrystals prepared by wet milling were orally administered to rats. The oral administration showed a five-fold increase of the meloxicam bioavailability in the presence of PVP compared to the coarse drug powder.

The possibility to combine the electrostatic and the steric stabilization approaches has also been proposed, for examples nanocrystal stabilized by SDS and Poloxamer have been described (71).

Lecithin was used in combination with poloxomer 188 to stabilize amoitone B, an anticancer agent.

The weight ratio (1:1) produced a stable formulation (72). The use of albumin or polymer derivatives of aminoacids for the nanocrystal stabilization has also been reported (73).

Other interesting strategies to stabilize nanocrystal have been developed: the use of various cyclodextrins without adding any surfactants, and the engineering of particle surface. Hong et al used 2-hydroxypropyl β-cyclodextrin alone or combined with other stabilizers to stabilize myricetin nanoscrystals (74). The Authors showed that the combination of 2-hydroxypropyl β-cyclodextrin with TPGS produced a superior stabilization than the cyclodextrin alone.

It is noteworthy that the presence of other excipients in the nanosuspension, such as preservatives, can modify the formulation stability by affecting the stabilizer agent's activity (23).

The possibility of producing self-stabilized nanosuspensions without stabilizers has also been investigated, although it is well known that stabilizers play a key role in preventing particle agglomeration .

Another stabilization strategy consists in the transformation of liquid nanosuspensions into solid forms, i.e. nanocrystal powders. Freeze-drying, spray-drying, pelletization and granulation are the solidification methods most widely used in the pharmaceutical field to obtain powders. These powders can be used as solid dosage forms as such, or as injectables after reconstitution or as oral powders. On the other hand, solid nanocrystals can be used to fill capsules, or they can be compressed into tablets preserving their properties. Moschwitzer proposed coated pellets as carriers of mucoadhesive nanocrystals of

hydrocortisone acetate surrounded with an enteric coating to achieve a controlled drug release (75).

Bulking agents, such as water-soluble sugars, are usually added to nanosupensions before the solidification process in order to maintain the physical-chemical properties of nanocrystals. Burgess and colleagues have recently evaluated the effects of a series of sugars and bulking agents in preventing nanocrystal aggregation during freeze-drying or spray-drying. They show that the combination of small sugars and polymers yields non-aggregate nanocrystal powders (76).

5. Applications of nanosuspensions

The characteristics of nanocrystals enable their administration by all the routes generally used.

Several research works focus on drug nanocrystal development for a specific route of administration. Some case studies are reported below.

5.1. Oral route

After oral administration, besides poor solubility and low permeability, another critical factor limiting absorption is the dissolution rate, i.e. the rate at which a solid drug or a drug in a formulation passes into solution (10). Since the drug's transit through the intestine occurs in a given time, the drug dissolution rate must be faster than the transit in order to maximize the absorption.

The most representative equation describing drug dissolution is the Noyes Whitney equation (27). The dissolution rate is a function of the concentration gradient across the layer, the width of the diffusion layer, the surface area of contact of the solid with the dissolution fluid and the diffusion rate of the drug in water. The concentration gradient in turn is a function of the maximum drug concentration at the surface of the dissolving solid and the concentration in the well-stirred bulk .

In particular for BCS class II compounds, the bioavailability may be enhanced by improving the solubility and dissolution rate of the drug in the gastro-intestinal fluids. The nanocrystal

technological approach makes it possible to obtain an increased drug dissolution rate and increased drug absorption in the gut lumen.

G.G Liversidge (77) compared a danazol nanosuspension, obtained with a ball milling process, with a conventional suspension of the drug, demonstrating that the nanosuspension increased oral bioavailability in beagle dogs. Moreover, the bioavailability of danazol nanosuspension was not significantly different from that of the cyclodextrin complex of the drug.

Moreover, Liversidge demonstrated that, by decreasing drug particle sizes in the submicron range and stabilizing the particles in suspension, it was possible to achieve a significant reduction of the gastric irritation caused by oral administration of naproxen. The reduced irritation and the improved rate of absorption were attributed to the decreased crystal sizes (78).

Jinno et al. (29) investigated the effects of particle size on the dissolution and oral absorption of cilostazol. They focused on several suspension formulations with different particle size distributions, prepared with various technological approaches from hammer-milling, jet-milling to NanoCrystal®. The results showed that the *in vitro* dissolution rate of cilostazol was significantly increased by reducing the particle size, indicating that the NanoCrystal® technology is an efficient means with which to improve the oral bioavailability of cilostazol and to avoid the food effect on the absorption.

The development of an oral rutin nanocrystal formulation has been particularly investigated. Mauludin din et al. (79) evaluated the possibility of obtaining dried rutin nanocrystals from a drug nanosuspension prepared with high pressure homogenization technology. Improved oral dissolution was observed on using dried rutin nanocrystals-loaded tablets. The improved physicochemical properties of dried rutin nanocrystals resulted in increased absorption and bioavailability in the gastrointestinal tract (71). In addition, rutin liquid nanosuspension has been investigated over time in order to assess its physico-chemical stability (80). The results showed that nanosuspensions obtained with high pressure homogenization can protect drugs from chemical degradation. In another

work the possibility of formulating quercetin, another flavonoid, as nanocrystals has been assessed .

Buparvaquone nanosuspension for oral administration has also been considered by Müller et al. (33). For this purpose, the nanosuspension was incorporated in mucoadhesive hydrogels, composed by different types of Carbopol® and chitosan, in order to increase the nanocrystals' adhesiveness in the treatment of the intestinal parasite *Cryptosporidium parvum*.

Another adhesive nanosuspension has been developed by Kayser et al. (81) by employing the high pressure homogenization technique to prepare amphothericin B nanocrystals with a mean diameter of about 500 nm. The *in vivo* efficacy of amphotericin B was improved with nanosuspension formulation.

Currently, several studies consider the possibility of modifying the nanocrystal surface in order to enhance their properties . To this end, Patel et al. (82) have developed an innovative formulation consisting of positively charged paclitaxel nanocrystals prepared by sonoprecipitation with a high pressure homogenization technique, and with an arginine-based surfactant as a stabilizer.

5.2 Parenteral route

Drug nanocrystals in suspension can be administered via several parenteral routes, from intravenous to intraperitoneal injections (22), because they can be sterilized by the current sterilization methods. Nevertheless, during the development of a parenteral nanosuspension, several requirements must be considered, more than in oral applications.

Firstly, the nanocrystals' dimensions must range below 5 µm to avoid capillary blockage. Moreover, only a few surfactants and polymers can be safely administered parenterally, making it difficult to stabilize a nanocrystals system; among them are lecithin, Poloxamer 188, Tween 80, low molecular weight polyvinylpyrrolidone (PVP) and sodium glycocholate. Several intravenous formulation developments have been reported. Moreover as already discussed, INVEGA SUSTENNA® is the only marketed product intended for parenteral use.

In order to deal with itraconazole's poor water solubility, Rabinow and co-workers investigated a tandem process of microcrystallization followed by homogenization to obtain an intravenous drug nanosuspension enhancing the efficacy of the antifungal agent (83) .

Möschwitzer et al. developed an intravenous omeoprazole formulation using the DissoCubes® technology. They achieved a more stable and high concentrated omeoprazole nanosuspension, thereby overcoming the chemical drug's degradability (84).

Danhier et al have recently investigated nanosuspension as a possible delivery method for an innovative poorly water soluble anti-cancer multi-targeted kinase inhibitor. They also compared nanosuspension formulation with other technological approaches, assessing its potentiality as a possible intravenous injection formulation and its higher efficacy (85). Liu et al. have recently studied the novel compound riccardin D, a potential poorly soluble anticancer drug. Both the bottom-up method and the top-down approach were used to develop an intravenous riccardin nanosuspensions(86). The following are other references on the development of intravenous nanosuspensions (87-89).

5.3 Pulmonary route

The pulmonary administration route has several advantages over other routes. It can be used to overcome first-pass hepatic metabolism, thereby reducing dose and side effects, and to treat respiratory disease locally. On the other hand, systemic treatment is possible as well, due to the large pulmonary surface areas and rich blood circulation which allow for fast drug absorption.

Nevertheless, nanoparticles in pulmonary delivery are still rare. Most of the published research focuses on inhaled drug nanoparticles deal with polymeric nanoparticles, liposomes, and solid lipid nanoparticles. There are only a few papers on the pulmonary delivery of drug nanocrystals. In order to produce an ideal respirable aqueous suspension, solid particles in the liquid dispersion should be below 1-5 µm.

Both pearl milling and high pressure homogenization technologies have been investigated to obtain pulmonary nanosuspensions.

For example, various studies report the processing of beclomethasone dipropionate with the ball milling approach (NanoCrystals™) to obtain stabilized nanosuspensions for pulmonary administration (90, 91). The possibility of obtaining a relatively stable aqueous hydrocortisone nanosuspension by bottom-up technology, using microfluidic reactors, has also been examined (92).

A budesonide nanosuspension for pulmonary administration was purposely tuned by Jacobs and coworkers (93). Challenging factors of this formulation were nebulization of the nanosuspension in order to permit administration to the lungs, and its long-term stability. Nanocrystal characterization before and after aerosolization confirmed suitability for pulmonary delivery.

Itarconazole, a new inhalation-optimized dry powder for the treatment of invasive pulmonary aspergillosis, has also been investigated by processing the drug with high pressure homogenization (94). For further details on the pulmonary delivery of nanosuspensions, the reader is referred to (95).

5.4 Topical route: from skin to ocular delivery

Generally, nanosuspension is considered suitable for dermal applications because of its ability to increase the penetration of poorly water soluble compounds into the skin. The first cosmetic product with rutin nanocrystals was marketed in 2007 (JUVEDICAL, Juvena Switzerland).

Nanosuspensions of the anti-oxidant resveratrol have been produced for dermal application by Kobierski et al. using high pressure homogenization (96). More recently, Shaal and co-workers have developed an hesperitin nanocrystals dermal formulation after investigating the influence of several preservatives on the stability of the product (97).

The ocular administration of poorly water-soluble drugs is considered a significant technological issue. The nanosuspension approach can increase corneal drug tissue levels, and thus bioavailability (98). Moreover, the reduced use of formulation additives makes nanosuspension a promising ophthalmic dosage form with which to decrease ocular irritation after administration. For this purpose, Kim and co-workers developed a novel

ophthalmic cyclosporin A-loaded nanosuspension using a top-down technique decreasing the ocular irritation in comparison with a commercial product (99) . Bottom-up approaches to obtain ocular nanosuspension have also been investigated. A diclofenac-loaded Eudragit S100-based nanosuspension was prepared by nanoprecipitation method by Ahuja et al. (100).

Recently the possibility to formulate dermal preparations based on nanocrystals of medium soluble molecules has been also investigated (101). In particular Zhai and colleagues proposed an innovative production process able to product physically stable nanocrystals from actives with medium solubility (23). In this research work the dermal administration of caffeine nanocrystals was studied, promoting the hair stimulation, by the beneficial nanocrystals accumulation in the hair follicles.

5. **Surface-modified nanocrystal formulations**

Nanocrystals have been also investigated as potential tool in the advanced nanomedicine field, as nanotechnological approach, not only for drug delivery but also for diagnosis (Figure 3). Thanks to this versatility, nanocrystals represent a good multitasking platform, able to evolve from the conventional to the innovative pharmaceutical formulations, through chemical modification of their surface.

First of all, due to their dimensions nanocrystals can be proposed as carrier-free drug nanodelivery systems, encapsulating over 99% of the active pharmaceutical molecule with a high ratio of drug to excipient (102). Moreover nanoparticles provides some benefits over microparticles, such as controlled drug release, modification of pharmacokinetics, biodistribution and cellular trafficking. Engineered formulations comprising surface-modified nanocrystals have been recently designed to improve their properties and biopharmaceutical parameters (103). Nanocrystals can be targeted in specific region of the body by the modification of their size and their surface. The localization of the drug at the disease site can improve its therapeutic index.

For instance surface-modified nanocrystals have been investigated in the anticancer field, where their small sizes may be exploited for passive targeting by the accumulation of

nanoparticles in tumor tissues. In fact, nanocrystals might reach the tumour site through the blood stream taking advantage of the enhanced permeability and retention (EPR) effect, due to the defective vascular architecture of the tumour tissue and poor lymphatic drenage (104).

Both coating and chemical conjugation approaches have been proposed to modify the nanocrystal surface (Figure 4). Staufenbiel and Müller developed an azithromycin nanocrystal formulation for i.v. administration by evaluating the influence of different coating agents on the protein adsorption patterns (105). Shubar developed surface-modified atovaquone nanocrystals for the treatment of toxoplasmosis infections by eradicating the parasite from the brain. For this purpose Apolipoprotein E, a protein with its receptor on the blood brain barrier (BBB), was attached to the nanocrystal's surface (106). Moreover the surface of atovaquone nanocrystals was also modified by the addition of poloxamer 188 and sodium dodecyl sulfate to improve oral bioavailability and passage through the blood-brain barrier, leading to an improvement in the treatment of toxoplasmic encephalitis and other cerebral diseases (107).

The surface of nevirapine nanocrystals has been modified with various substances, such as albumin, dextran and poly(ethylene)glycol (PEG). The albumin-coated system exhibited a four-fold increase in macrophage uptake, in respect to the free drug. Shegokar and co-workers demonstrated that the in vivo biodistribution of surface-modified nevirapine nanocrystals increased the drug accumulation in various organs, such as liver, spleen and brain, compared with drug solution after intravenous administration to rats (108).

Wang developed docetaxel nanocrystals coated with soy lecithin showing an increased accumulation of the drug at the tumor site after intravenous adminstration to rats (109).

Moreover the nanocrystal surface can be modified with fluorescent molecules to obtain a labelled system for diagnostic purposes. Recently, Hollis et al designed nanocrystals in which imaging agents are incorporated in the crystal lattice obtaining hybrid nanocrystals (110). They can be considered a theranostic systems permitting simultaneously an anticancer treatment and the imaging of the real-time evaluation of the nanocrystal biodistribution. For this purpose tritium-loaded paclitaxel nanocrystals with an optical dye

physically entrapped in the structure were prepared by nanoprecipitation spiking the drug solution with the imaging probe. Subsequently the nanocrystals underwent to homogenization to achieve size uniformity. These hybrid nanocrystals were injected intravenously in a mouse xenograft model. Whole-body optical imaging of animals was carried out. It was found that only 1% of the injected dose was accumulated in the tumor tissues.

Finally, selective ligands for active targeting can be linked to nanocrystals (111). Leroux and colleagues prepared targeted nanocrystals by the chemical bonding of crosslinkable polymers on the nanocrystal surface. Briefly, functional copolymers bearing alkynyl or azido groups which may prevent aggregation and alter circulation time of drug nanocrystals by "click-chemistry" mediated cross-linking (112).

Liu prepared targeted paclitaxel nanocrystals (102) by conjugating a folic acid ligand to Pluronic F127 used as nanoparticle coating obtaining a new nanomedicine formulation with a target therapeutic effect.

6. Products on the market and conclusions

In regard to nanocrystal formulations currently on the market, the most significant products have been developed for oral administration because of its easier requirements compared with the parenteral route. Most of the nanocrystal products are obtained using the pearl mill technology developed by Elan Nanosystems (24). In 2000 the first formulation Rapamune® was placed on market by Wyeth Pharmaceutical. It consists of nanocrystal tablets containing sirolimus in a low dose: nanocrystals are released from tablets as an ultrafine suspension. Merck introduced the second product Emend® in 2001: it consists of an aprepitant hard gelatin capsule filled with a single drug nanocrystal dose incorporated into pellets.

In 2004 the FDA approved the TriCor®, fenofibrate tablets for hypercholesterolemia marketed by Abbott Laboratories. Fenofibrate nanocrystals are also formulated as tablets in the SkyePharma Triglide: in this case nanocrystals are obtained with IDD-P® technology and marketed by Sciele Pharma Inc. (Atlanta, CA, USA).

Megace ES® (where ES stands for enhanced solubility) developed by Par Pharmaceutical Companies Inc., is a liquid oral nanosuspension for the delivery of megestrol acetate, an antianorexic agent. Megace ES has improved the original conventional Megace product by utilizing NanoCrystal® Technology (113). Megace ES can be administered in a smaller volume with a viscosity lower than conventional Megace suspension. This may be important for patients affected by loss of appetite, who typically have difficulty in swallowing (114).

As regards nanocrystal pharmaceutical dosage forms different from oral administration, INVEGA® SUSTENNA® (paliperidone palmitate) has been available since 2009 (Janssen). This is an extended-release injectable suspension for schizophrenia treatment in adults. INVEGA SUSTENNA® was developed by utilizing NanoCrystal® Technology (Elan Drug Technologies' proprietary), and it is an aqueous nanosuspension for once-monthly intramuscular administration which provides an increased rate of dissolution. INVEGA SUSTENNA® is supplied as a pre-filled syringe which does not require reconstitution or refrigeration. After the administration, it acts as a drug reservoir providing an extend release over time.

These are the nanocrystal-based products on the market, but it should be emphasised that several products are now in the pre-clinical and clinical phases and are expected to reach the market in the next few years. This tendency underlines the concept that drug nanocrystals are one of the most promising approaches in the delivery field to improve the administration of drugs. This formulation method may be also applied to obtain nanomedicine products with easy manufacturing processes and further potential modification for targeting.

7. Expert Opinion

Currently much research is focused on the development of drug nanocrystals, seeking updated products and new engineered nanocrystal formulations.

Drug nanosuspensions can be considered versatile delivery platforms combining traditional and innovative pharmaceutical products. Their distinctive advantage is the capacity to solve biopharmaceutical issues, such as low bioavailability after oral or dermal administration, and the potential to decrease the amount of the drug administered and thus reduce adverse side effects.

Drug nanocrystals can be easily translated from lab-scale to industry scale by using conventional equipment and preparation methods. As such, they can be a user-friendly liquid product or a component of conventional dosage forms.

The tunable doses and the easy oral administration of drug nanosuspensions make them particularly interesting for the preparation of pediatric formulations and for patients with swallowing difficulties. Moreover nanocrystals possess a meal-independent drug absorption, reducing bioavailability between fed and unfed conditions. Indeed, the adhesive properties of nanocrystals play a key role in this mechanism by making nanocrystal formulations not sensitive to the food effect.

The key role in nanocrystal stability is played by the addition of stabilizers. Alternatively, to improve stability, nanocrystals can be freeze-dried to obtain a powder that can be used as a component of conventional dosage forms such as tablets or capsules.

On the other hand, nanocrystals can be considered as nanoparticulate carriers with the relative advantages.

Nanocrystals may be considered superior to other colloidal drug delivery systems due to their composition, consisting 100% of pure drug, which reduces excipient use and the potential safety issues related to them. They can overcome the limitation of a low drug payload and modify pharmacokinetic parameters and biodistribution.

The adhesiveness of nanocrystals should be emphasised. Their strong adhesion capacity is due to their increased contact area provided by their higher surface area and van der Waals

interactions. This behaviour can be exploited both in oral administration, to increase absorption through the gastro-intestinal tract, and in local administration, dermal, ocular or pulmonary, to achieve a more effective and sustained therapeutic response due to the prolonged contact time of the drug at site of action.

The intrinsic adhesiveness capacity can be improved by coating nanocrystals with certain polymers. In recent years also the possibility of engineering the nanocrystal's surface has been investigated, the purpose being to improve the in vivo performance of nanocrystals or to enhance their physico-chemical stability over time, or for targeting purposes.

Nevertheless, the nanoparticle-like behaviour of drug nanosuspensions also has some drawbacks especially related to potential nanotoxicity after their administration. In fact, drug nanocrystals may easily enter into cells, because their sizes regulate the uptake of particles. Moreover, their complete and very rapid dissolution may cause negative systemic effects in the organism.

Anyway nanocrystals are considered a low risk class for sizes and dissolution rate (37). Nevertheless, nanomedicine formulation will grow in the future, and drug nanocrystals can represent the 'zip tool' between the traditional pharmaceutical formulations and the innovative ones. To date, nanocrystal technology has been mainly investigated for the delivery of poorly-water soluble drugs, because of their bioavailability limitations affecting their in vivo performance, but they can be used to produce commercial products.

In the future the application of nanocrystal technological approach might be deeply investigated in order to include also the formulations based on medium water-soluble drugs and biological molecules, with the aim to improve their therapeutic efficacy.

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Tables

Measured parameters	Techniques
Particle size and size distribution	PCS/DLS Laser diffraction Coulter counter
Particle size and morphology	SEM/TEM AFM
Sedimentation/creaming	Visual observation/ laser backscattering/ near infrared transmission
Particle surface charge/zeta potential	Laser Doppler electrophoresis
Crystallinity state	XRD/DSC
Chemical stability	HPLC/FTIR/NMR/MS

Table 1 Main Techniques used in the physico-chemically characterization of nanosuspension formulations

Figures legends

Figure 1: General overview of the main properties of nanosuspensions as versatile drug delivery platform

Figure 2 Main approaches used in the nanosuspensions production, either at lab-scale or on an industrial scale, classified into bottom-up and top-down technologies.

Figure 3 Schematic overview of the nanocrystal applications as a versatile zip tool, useful to link traditional pharmaceutical formulations and nanomedicine

Figure 4 Schematic representation of a coated nanocrystal and a engineered nanocrystal, representing the third generation of nanocrystals